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RELEASE AND ESTABLISHMENT OF DIFFUSE AND SPOTTED KNAPWEED BIOCONTROL AGENTS BY USDA, APHIS, PPQ, IN THE UNITED STATES

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Abstract.—Spotted and diffuse knapweed are major weed pests of rangeland in the Western and Midwestern United States. Canada, United States university experiment stations, United States Department of Agriculture, Agriculture Research Service (ARS), and local weed control groups began an effort to introduce biological control agents for spotted and diffuse knapweed in early 1970 (Table 2). APHIS perceived the need to respond and organize a regional effort to an enormous weed problem. They set up rearing procedures, protocols, established a structure for distribution, and established a protocol for monitoring the establishment and effect of the introduced biological control agents. In 1987, the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection Quarantine (USDA, APHIS, PPQ) in cooperation and consultation with other interested biological weed control groups began a biocontrol program against spotted and diffuse knapweed with the release of three biocontrol agents. By 1998, 13 knapweed biocontrol agents had been released in 17 states and 112 counties. At least one agent has established in each state where introductions were made. Eleven of the 13 agents released by APHIS have become established. Nine of the 13 biocontrol agents are now collectable in some states. *Agapeta zoegana* is collectable in eight states, *Cyphocleonus achates* in six states, *Larinus minutus* in ten states, *Metzneria paucipunctella* in three states, *Sphenoptera jugoslavica* in eight states, *Terellia virens* in one state, *Urophora affinis* in eight states, *Urophora quadrifasciata* in ten states and *Larinus obtusus* is ready to be collected in one state.

Key Words.—Insecta, biocontrol, insect, *Centaurea,* knapweed, spotted, diffuse.

Diffuse and spotted knapweed (*Centaurea diffusa* Lamarck and *C. maculosa* Lamarck) are Eurasian plants that have become serious weed pests of rangeland, pastures and waste areas (Roché & Talbott 1986, Watson & Renney 1974). Diffuse knapweed is a biennial and spotted knapweed is a short lived perennial (Watson & Renney 1974). *Centaurea diffusa* was first discovered in Washington in 1907 and *Centaurea maculosa* was found in Washington in 1923 (Roché & Talbott 1986).

A 1994 study of the economic impact of knapweed spp. in Montana found that the 805,600 hectares of knapweed infested land caused a loss of 42,107 million dollars annually; the equivalent of 518 jobs (Hirsch & Leitch 1996).

Since 1988, United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection Quarantine (USDA, APHIS, PPQ) has coordinated implementation of a classic biocontrol program against spotted and diffuse knapweed in the United States.

APHIS has implemented a three phase strategy to establish and redistribute biocontrol agents of spotted and diffuse knapweed. Phase I is the introduction of approved biocontrol agents, often through quarantine facilities, from foreign sources (including Canada). The purpose of this initial phase is to establish field

insectary sites (FIS). A FIS is defined as "a weed infested location that will be managed to produce insects for eventual redistribution to other weed infested sites" (Hansen et al. 1997). Each FIS was chosen with regard to conditions that optimize the chances of establishment of the biocontrol agent.

Phase II serves to increase the number of FIS from agents reared in the original Phase I FIS and to involve state cooperators in the management and maintenance of the FIS. Phase III begins when these insectaries reach collectable population size and the collection and the redistribution of beneficial agents becomes the responsibility of federal, state, county, and local cooperators in each state.

APHIS has released 13 biocontrol agents for the control of spotted and diffuse knapweed (Table 1). These agents have been tested for host specificity by the International Institute of Biological Control (IIBC), European Station, Delmont, Switzerland or the United States Department of Agriculture Research Station, European Biological Control Laboratory (Montpelier, France) (ARS) before being approved for importation and release in the United States. The tests have shown that these agents have narrow host ranges. The biocontrol agents are: *Agapeta zoegana* (L.) (Lepidoptera: Cochylidae) (Müller et al. 1988), *Bangasternus fausti* Reitter (Coleoptera: Curculionidae) (Sobhian et al. 1992), *Chaetorellia acrolophi* (White & Marquardt) (Diptera: Tephritidae) (Groppe & Marquardt 1989), *Cyphocleonus achates* Fabr. (Coleoptera: Curculionidae) (Stinson 1987), *Larinus minutus* (Gyellenhal) (Coleoptera: Curculionidae) (Groppe 1990), *Larinus obtusus* Gyellenhal (Coleoptera: Curculionidae) (Groppe 1992), *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae) (Englert 1973), *Pelochrista medullana* Staudinger (Lepidoptera: Tortricidae) (Gassmann et al. 1982), *Pterolonche inspersa* Staudinger (Lepidoptera: Pterolonchidae) (Dunn et al. 1989), *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae) (Zwöolfer 1976), *Terellia virens* (Loew) (Diptera: Tephritidae) (Groppe & Marquardt 1989b), *Urophora affinis* Frauenfeld (Diptera: Tephritidae) (Zwölfer 1970), and *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) (Rees & Story 1991).

The purpose of this paper is to document the release, recovery, and establishment of the thirteen biocontrol agents (insects) that have been released in the United States by APHIS. These agents have been released in cooperation with federal, state, county, and local agencies as well as individuals where appropriate.

Biologies of Released Agents

Agapeta zoegana, a univoltine moth, begin to mate within twenty-four hours of emergence. Within twenty-four hours after mating *A. zoegana* females begin to oviposit on the rosette leaves of spotted and diffuse knapweed. The neonate larva moves immediately to the root crown of the young knapweed rosette and begins to move down into the root system completely eating smaller roots and damaging the cortical tissue of the larger roots. The larvae feed just under the outer layer of root tissue near the crown or up to 4 cm down in the root. This mining either kills or weakens and stunts the knapweed plant. When small rosettes are killed the larvae are capable of moving up to 10 cm and attacking another knapweed plant (Müller et al. 1988).

Bangasternus fausti is an univoltine weevil. The adults overwinter in the debris on the soil or in the seedheads, emerge in early spring, and immediately begin copulating and ovipositing. The female feeds on the flower and lays her egg on

the clipped surface. She may also lay her eggs on the bracts of the flower head or on the terminal part of the stem always covering the eggs with an exude from her anus. The larvae emerge from the eggs in eight to twelve days, then mine through the stems directly into the flower bud. Generally there will be one larva per seedhead (Sobhian et al. 1992). The new adults emerge from the seedheads in late summer through fall.

Chaetorellia acrolophi is a facultative bivoltine seedhead fly that attacks spotted knapweed with two overlapping generations in midsummer. Adults emerge from last year's seedheads in July and begin mating. The eggs are laid under the bracts of closed flower buds. The neonate larvae burrow horizontally through the bracts and some florets until they reach the center of the bud. The larvae feed on the achenes, florets, and some on the receptacle through the second and third instar destroying the seedhead contents (Groppe & Marquardt 1989).

Cyphocleonus achates is an univoltine weevil, which emerge from the roots from late July to late September. After emergence, the adults feed on the leaves preferring tender leaves at the center of the rosette. The adults mate several times during the ten week oviposition period. The eggs are laid in the root crown. Larvae emerge in ten to twelve days, mining through the root crown into the tap root. There may be multiple larvae in the same knapweed root. Larvae overwinter as second instar and begin feeding and complete development the following spring (Stinson 1987).

Larinus minutus is an univoltine weevil with adults emerging from the seedhead in late September to overwinter in the debris on the soil. Spring emergence of adults begins from late May through mid June. Oviposition occurs after the knapweeds have begun to flower as the females must feed on the freshly opened flowers for their ovarioles to develop. The adults primarily feed in the mid-region of the capitula while preparing the area for oviposition. The eggs hatch in three days and the larvae pupate in about four weeks. The larvae consume the pappus hairs, then the achenes, and often attack the receptacle. One larvae develops per seedhead in diffuse knapweed and one to four larvae developing in spotted knapweed seedheads (Groppe 1990).

Larinus obtusus is an univoltine weevil that feeds on and oviposits in spotted knapweed flower heads. The adults emerge from the seedheads in late summer to early fall, feed on the knapweed leaves and then diapause in the debris and soil around the base of the plants. In the spring, the adults emerge and feed on the knapweed leaves until flowers become available. Oviposition takes place in the open flower heads in the middle part of marginal florets. The female feeds on the florets while preparing the oviposition site and letting her ovarioles mature. The oviposition period may last from seven to ten days. Newly hatched larvae feed downward into the flower, eating seed and pappus hairs. Mature larvae form a cavity in the flower head making a pupal case by cementing seed, pappus hairs, and frass together in a hard chamber like structure (Groppe 1992).

Metzneria paucipunctella is an univoltine moth that attacks spotted and sometimes diffuse knapweed seedheads. Oviposition starts two to three days after adult emergence from last years seedheads. The eggs are laid at the base of or on the stem just below an unopened flower head. The newly hatched larvae climb the flower bud and enter the open flower, first feeding inside the tubular flower, and after molting feeding inside one or two immature achenes. Before the next moult

the entrance hole is closed with a silken web. The third instar larvae emerge from the achene and tunnel through the receptacle, destroying and penetrating several achenes. The fourth instar feeds laterally through a group of achenes bound together by a web. The last instar builds a tunnel like web through the flower that serves as an exit hole for the moth (Englert 1973).

Pelochrista medullana is an oligophagous, univoltine root feeder. Mating takes place in daylight within twenty-four hours of emergence at temperatures of 18 to 30° C. Oviposition begins two to three days after adult emergence with eggs laid singly or in batches of two to three eggs on the plant crown leaves. The ideal temperature for maximum egg production and viability is 18.4° C. Larvae hatch in seven to ten days, move to the center of the rosette and mine into the root. They feed through the fall and diapause through the winter, and resume feeding in the spring. Roots of smaller plants are entirely destroyed, but roots less than five millimeters in diameter will not support larva to maturity. Larva on smaller plants often die because they lack the ability to move to another plant (Gassman et al. 1982).

Pterolonche inspersa is a univoltine, root boring moth. Larval feeding takes place for up to 11 months out of the year. In July and August the adults exit through the root crown, emerging through a pre-made silken tube in the feeding area. Adults begin mating in the afternoon of emergence with oviposition peaking in late evening. The eggs are laid singly or in groups on the leaves of rosettes, older plant leaves and stems of mature plants. The larvae hatch and immediately move to and mine into the root crown making the silken tube as they feed. Mature larvae pupate in the root and emerge as adults in about 14 days. The adults live 10 to 15 days. Larval feeding causes the knapweed roots to become soft and prone to disease. The feeding damage reduces the storage capacity of the root making it difficult for the plants to overwinter (Campobasso et al. 1994).

Sphenoptera jugoslavica is univoltine with a small percentage of adults emerging the following year. Adults emerge from June to July, mating on the third day after emerging. They repeatedly mate for the next 30 days. Eggs are generally laid at the base of the plant on the under side of the leaves. Neonate larvae burrow into the plant and down into the root. To be successful, egg laying and larval emergence must coincide with a period of arrested rosette growth. The larvae cease feeding in the winter and resume in the spring, pupating from mid June to mid July. Each plant generally supports one larva (Zwölfer 1976).

Terellia virens, a bivoltine seedhead fly with adults found in the field from early June through August. The female oviposits in young opening flowers, depositing the egg between the florets. The larvae hatch in three to five days, developing and pupating in about fourteen days. The larvae live in a single seed through the third instar. They then feed on the seed itself damaging the germ of older seed. The second generation larvae diapause as a prepupae and pupate in the spring (Groppe & Marquardt 1989b).

Urophora affinis is a univoltine gall forming seedhead fly. The adults begin to emerge in June. Mating begins soon after emergence and lasts for up to three weeks. Eggs are laid singly or in a small group on unopened seedheads. The larvae emerge from the eggs in three to four days. The larvae penetrate the ovariole of the undeveloped tubular flower causing the formation of a fusiform gall

in the receptacle which destroys the achenes and deforms the receptacle. The larva diapause in the gall (Zwölfer 1970).

Urophora quadrifasciata is a bivoltine, gall forming seedhead fly. The adults appear in June in Montana and a partial second generation may be found in August. Females begin oviposition on the second or third day after emergence, laying eggs singly among the stamens of the flower. In three to four days the larvae hatch and burrow into the ovary. A gall begins to form in eight days causing the ovary cells to multiply and form nutritive tissue which reaches maximum size in fifteen days. This gall, unlike the *U. affinis,* is not lignified and the larvae will consume nearly the entire gall destroying the floret. The second generation larvae over winter in the gall as prepupae and pupate in the spring (Harris 1986).

Materials and Methods

Agapeta zoegana.—APHIS procured *A. zoegana* from many sources, IIBC in Switzerland, Agriculture Canada, and Montana State University Research Station in Corvallis, Montana. *Agapeta zoegana* first were mass reared in insectary gardens consisting of cages 3 × 3 × 2.34 m (standard-sized cages) (Story et al. 1994) and when *A. zoegana* populations established, the moths were collected directly from field insectaries. *Agapeta zoegana* were collected from field and cage insectaries with a modified insect vacuum from the plants during the day (Powell et al. 2000). Black lighting on a moonless, warm, still night, with a white bedsheet attached between two posts or trees was also used to collect this agent (Fitzpatrick 1989). The modified vacuum was developed by Alan Sturko of Agriculture Canada and further modified by APHIS (Story et al. 1994, Powell et al. 2000).

Open field releases of 50 to 100 plus and cage releases of 25 adult moths were recommended in standard-sized cages. Cages 0.762 × 0.762 × 0.762 m (small cage) were used for releases of 10 to 12 moths. Small cage releases consisted of a minimum of two cages per release and were used in hard to access sites or when small numbers of *A. zoegana* were available. The cages were removed 5 to 10 days after the release was made. *Agapeta zoegana* adults were released on the lower part of the plants, in the evening or cooler part of the day so that the moths would settle on the plants and remain in the area.

Agapeta zoegana were monitored visually by checking knapweed plants for moths and by using a black light to attract them to a white surface (Fitzpatrick 1989). Caged releases were monitored by looking on the cage screen walls for resting adults. Field recovery in the daytime consisted of looking for the moths resting on the knapweed leaves or stems (Story et al. 1994).

Bangasternus fausti.—Bangasternus fausti were procured from ARS collections originating in European field insectaries. *Bangasternus fausti* were field released as adults on one to two hectare plots of spotted or diffuse knapweed. A sweep net was used to collect *B. fausti* in the morning when the temperature began to rise. One hundred plus *B. fausti* adults were recommended for field releases. The adult weevils were scattered within a 1.5 m diameter circle around a central stake.

Monitoring to check for presence and establishment of *B. fausti* was done by examining bolted plants for feeding adults on the new formed buds during the warmer part of the day (23° C) (Sobhian et al. 1992). Sweeping was used as a

monitoring tool after the knapweed plants had bolted and before the plants exceeded 10 percent bloom.

Chaetorellia acrolophi.—Chaetorellia acrolophi were shipped to the United States through quarantine from European sources by IIBC, Delmont, Switzerland. Standard-sized cages in gardens and later, the field were used for mass rearing of *C. acrolophi* (Groppe and Marquardt 1989). Adult *C. acrolophi* were collected for shipment and redistribution from standard-sized cages containing infested bouquets of spotted knapweed from caged garden plots. The adults were collected daily from the sides of the cages with a modified insect vacuum (Powell et al. 2000).

Minimum cage releases for *C. acrolophi* were 50 adults per standard-sized cage. *Chaetorellia acrolophi* were monitored for presence and establishment by seedhead dissection checking for larval presence in the seedheads (Groppe and Marquardt 1989). The seedheads were collected after a killing frost and the plants had matured and dried out. Two hundred seedheads were collected from the field release sites by walking in concentric circles and taking two seedheads from individual plants. Fifty of the seedheads were selected at random and dissected to check for larval density and presence. Caged insectaries were monitored for *C. acrolophi* presence by checking for *C. acrolophi* presence by checking for emerging adult files on the sides of the cages. This agent was limited in numbers so no destructive sampling method was used in cage releases to determine larval presence.

Cyphocleonus achates.—Cyphocleonus achates were shipped into the United States quarantine from European collection sites by IIBC, Delmont, Switzerland. Agriculture Canada also supplied some *C. achates* adults to APHIS to establish insectaries in the United States. *Cyphocleonus achates* were mass reared in spotted knapweed gardens (Story et al. 1996).

Cyphocleonus achates adults were collected by hand picking from the plants in the field or garden. *Cyphocleonus achates* releases of 50 plus were used and two or three adult weevils were placed on each knapweed plant keeping the total release within 5 meters of the central stake.

Cyphocleonus achates were monitored by visually checking for adults on rosettes or blooming mature plants from the first part of August to late fall. *Cyphocleonus achates* adults tend to go to the tops of the plants during the heat of the day.

Larinus minutus.—Larinus minutus were procured from Europe through IIBC. Field insectaries were used to mass rear *L. minutus*. *Larinus minutus* were field released as adults in one to two hectare plots of spotted or diffuse knapweed. *Larinus minutus* adults were collected with a sweep net in the morning as the temperature began to rise. *Larinus minutus* were released in numbers of 100 plus and scattered within a 1.5 meter diameter circle around a central stake.

Monitoring to check for presence and establishment of *L. minutus* was accomplished in the early spring by checking rosettes for feeding adults. The rosettes were examined by carefully lifting the leaves, visually checking for adults feeding under the leaves or physically pulling and shaking the rosettes over a white cloth to catch falling adults (Lang et al. 1996). Sweeping was used as a monitoring tool after the knapweed plants had bolted and before they had exceeded 10 percent bloom. Monitoring also took place after the flowers were in full bloom by check-

ing blossoms for feeding adults. *Larinus minutus* presence and establishment was also monitored by looking for emergence holes in the open seedheads in the winter and late fall (Groppe 1990).

Larinus obtusus.—Larinus obtusus were originally procured for European insectaries through IIBC, Delmont, Switzerland. This agent was mass reared in field insectaries. *Larinus obtusus* were field released as adults on one to two hectare plots of spotted or diffuse knapweed.

Larinus obtusus were collected with a sweep net or from the individual spotted knapweed flowers in the morning as the temperature began to rise. *Larinus obtusus* were released in numbers of 100 plus and scattered within a 1.5 m diameter circle around a central stake.

Monitoring to check for presence and establishment of *L. obtusus* was accomplished in the early spring by checking bolted plants with newly formed flower buds for feeding adults both visually and using a sweep net (Groppe 1992). Monitoring also took place after the flowers were in full bloom by checking blossoms for feeding adults.

Metzneria paucipunctella.—Metzneria paucipunctella were procured from Agriculture Canada. Mass rearing of *M. paucipunctella* were in field insectaries. *Metzneria paucipunctella* were field released as adults on one to two hectare plots of spotted knapweed.

Adult *M. paucipunctella* were collected for shipment and redistribution from standard-sized cages containing infested spotted knapweed bouquets or seedheads. The adult moths were collected daily from the sides of the cage with a modified insect vacuum (Powell et al. 2000, Story et al. 1991). *Metzneria paucipunctella* adults were released on the lower part of the plants in the evening or cooler part of the day so that the moths would settle on the plants and remain in the area. Local releases of *M. paucipunctella* consisted of a minimum of 10 bouquets of moth infested mature spotted knapweed plants per release at the site to be infested. Recommendations of 500 adult moths were used for interstate redistribution.

Metzneria paucipunctella were monitored by dissecting seedheads to check for larvae of the agent (Englert 1973). Two hundred spotted knapweed seedheads were collected after a killing frost and the plants had matured and dried out. The seedheads were collected from a release site by walking in concentric circles and taking two seedheads from individual plants. Fifty of these seedheads were selected at random and dissected to check for larval density and presence.

Pelochrista medullana.—Pelochrista medullana were supplied from European field collections by IIBC, Delmont, Switzerland. The majority of these insects were released as neonate larvae on knapweed rosettes. No collecting method was developed by APHIS.

This agent was monitored by placing a cage over the infested rosettes in the spring following the initial larvae releases (Gassman et al. 1982). The sides of the cages were checked daily in the spring and summer for adult moths.

Pterolonche inspersa.—Pterolonche inspersa were obtained from European field collections through IIBC, Delmont, Switzerland. This agent was released as neonate larvae on spotted knapweed rosettes. No collecting method was developed by APHIS.

This agent was monitored by placing standard-sized cages over the rosettes the following spring after the larval release (Campobasso et al. 1994, Dunn et al.

1989). The sides of the cages were checked daily for adult moths throughout the spring and summer.

Sphenoptera jugoslavica.—Sphenoptera jugoslavica were procured from Agriculture Canada for mass rearing in field insectaries. *Sphenoptera jugoslavica* were released as adults in field insectaries on one to two hectare plots of diffuse knapweed.

This agent was collected with sweep nets in early evening as the temperature began to drop. Sweeping for *S. jugoslavica* usually began at 1800 hours. Collection for this beetle began when the diffuse knapweed reached 10 percent bloom. Field releases of 500 adult *S. jugoslavica* were recommended by APHIS.

Sphenoptera jugoslavica presence and establishment was detected by sweeping for adults when the diffuse knapweed was at 10 percent bloom or by digging plants near the release point in March and checking the roots for larval presence. Infested roots are swollen just below the crown and when cut open the *S. jugoslavica* larva is visible (Zwolfer 1976).

Terellia virens.—Terellia virens were obtained from European field collections through IIBC. Standard-sized cages were used to assist the recovery and mass rearing of *T. virens. Terellia virens* were also field released as adults on one or two hectare plots of spotted knapweed.

Terellia virens were collected from standard-sized cages containing infested bouquets or seedheads. Adults were collected daily from the sides of the cages with a modified insect vacuum (Powell et al. 2000, Story et al. 1994). Minimum releases of *T. virens* were 50 adults per cage and up to 500 adults for field release. *Terellia virens* were monitored by spotted knapweed seedhead dissection checking for larval presence (Groppe and Marquardt 1989b). The spotted knapweed seedheads were collected after a killing frost and the plants had matured and dried out. Two hundred seedheads were collected from the release sites by walking in concentric circles and taking two seedheads from individual plants. Fifty of these seedheads were selected at random and dissected for larvae presence and density.

Urophora affinis and Urophora quadrifasciata.—Urophora quadrifasciata dispersed from Canada into the United States (Gillespie 1983, Story et al. 1987). Montana State University Research Station released the first *Urophora affinis* in Montana in 1973 (Story 1984) (Table 2). APHIS collected *U. affinis* and later *U. quadrifasciata* from the local populations in Montana for redistribution throughout the western and midwestern states (Lang et al. 1997). *Urophora affinis* and *U. quadrifasciata* were redistributed locally by collecting and moving *Urophora* fly-infested spotted knapweed bouquets (Story 1984). Bouquets averaging 500 seedheads were collected from spotted knapweed infestations where the *Urophora affinis* population averaged greater than 1.5 *Urophora* galls per seedhead. The knapweed bouquets were tied to stakes, trees, or posts (Story 1984). *Urophora* flies emerging from the seedheads were synchronized with the development of the knapweed plants (Story 1984). Up to 200 bouquets were placed in cages to rear out adult *Urophora* flies for interstate shipments (Lang et al. 1997). Adult *Urophora* flies were collected daily for shipment and redistribution from the standard-sized cage walls with an insect vacuum. It was recommended that a minimum of 10 *Urophora* infested bouquets or 1000 *Urophora* adults be released at each release site.

Urophora affinis and *U. quadrifasciata* were monitored for establishment and

presence by seedhead dissection checking their distinctive galls formed in the seedheads for the larvae (Harris 1986, Nowierski and Story 1988). The seedheads were collected after a killing frost and the plants had matured and dried out. Two hundred seedheads were collected from the release site by walking in concentric circles and taking two seedheads from individual plants. Fifty of these seedheads were selected at random and dissected to check for gall and larvae density and presence.

All agents were shipped for redistribution using overnight shipping services, in one quart cardboard cylindrical containers packed in insulated shipping boxes with frozen blue ice. The blue ice was separated from the agent containers with styrofoam beads.

Field releases consisted of putting a specified number of agents at a site. Generally, the best sites were patches of knapweed plants that were not solid monocultures, but had some open areas among the plants. South-facing slopes were chosen when available to gain as many degree days as possible. A permanent stake was used to mark the point of release.

As a general rule, spotted and diffuse knapweed agents were considered established if recovery was made in the second year after the initial release (Lang et al. 1996, 1997, 1998).

Results and Discussion

Biological control agents for the control of spotted and diffuse knapweed have been released in seventeen states and one hundred-twelve counties since 1988 (Table 1). At least one biocontrol agent has established in each of the seventeen states. Some states did not have suitable conditions for certain control agents. For example, *M. paucipunctella* cannot tolerate temperatures below minus 22° F (Good et al. 1997). *Cyphocleonus achates* need an average of 2320 degree days to complete development (Hansen, unpublished data), and *C. acrolophi, T. virens,* and *L. obtusus* need spotted knapweed heads to develop. The agents that were shipped to the different states were selected to accommodate the needs of the state and conditions for establishment of the biocontrol agents.

The reproductive capacity, ease of establishment and length of time since introduced into the United States influence the current range of the individual agents. The 13 biological control agents for diffuse and spotted knapweed were introduced over a 19 year period (Table 2). APHIS joined the biological control of spotted and diffuse knapweed effort beginning in 1987 and began a multiple state release program for the biological control agents as they became available (Table 2). State universities, federal government agencies such as ARS, and state and local weed control boards are also involved in the biological control program for spotted and diffuse knapweed.

For detailed information on *U. affinis* and *U. quadrifasciata* release and establishment status refer to Lang et al. (1998).

Agapeta zoegana is collectable and in Phase II and III in eight states, Phase II in Minnesota, Phase III in Colorado, Montana, Oregon, South Dakota, Utah, Washington and Wyoming. *Chaetorellia acrolophi* has been recovered from caged releases and redistributed in Colorado, Minnesota, and Montana and field recoveries have been made in Oregon. *Cyphocleonus achates* has been released, established in, and is collectable in Colorado, Montana, Oregon, Utah, Washington,

Table 1. The status of biocontrol agents for spotted and diffuse knapweed released by USDA, APHIS, PPQ

State	Agent	County	Year/years released	Status
ARIZONA	*A. zoegana*	Coconino	92, 93	N
		Gila	94	N
	L. minutus	Gila	95	N
	S. jugoslavica	Coconino	93, 97	N
		Gila	94	N
CALIFORNIA	*L. minutus*	Shasta	95	Unk
		Trinity	95	Unk
	T. virens	Shasta	95	Unk
COLORADO	*A. zoegana*	Arapahoe	91	N
		Douglas	91	N
		La Plata	94–95	N
		Larimer	92	E
		Mesa	93	E
		Montrose	94–96	N
	B. fausti	Douglas	93–94	N
		La Plata	94	N
		Montrose	95	N
	C. acrolophi	Mesa	96–97	E
		La Plata	97	N/A
	C. achates	Archuleta	94	D
		Clear Creek	94	N
		Douglas	94, 96	N
		El Paso	95–97	R
		Gilpin	95	R
		Jefferson	94, 96	N
		La Plata	93–95, 97	E
		Larimer	94	R
		Mesa	92–93, 97	E
		Montrose	94–97	N
	L. minutus	La Plata	95, 97	N
		Mesa	97	N/A
		Montrose	95, 97	R

Table 1. Continued.

State	Agent	County	Year/years released	Status
	L. obtusus	Montrose	96	Unk
		El Paso	97	N/A
	M. paucipunctella	Douglas	93	N
		La Plata	92–93	E
		Larimer	92	R
		Montrose	93	N
	P. inspersa	Mesa	95–97	N
	S. jugoslavica	Archuleta	91	D
		Arapahoe	95	N
		Boulder	91, 97	E
		Douglas	91, 93–95	E
		E Paso	96	N
		Freemont	97	N/A
		Jefferson	92, 94–97	E
		Logan	96	N
		Montrose	96	N
	T. virens	La Plata	95	N
		Mesa	96	N
IDAHO	*A. zoegana*	Benewah	94	Unk
		Blaine	94	Unk
		Boise	94	Unk
		Bonner	94	Unk
		Idaho	94–95	Unk
		Jefferson	93	Unk
		Lemhi	95	Unk
		Shoshone	93	Unk
	B. fausti	Boise	94	Unk
	C. achates	Clark	95	Unk
		Clearwater	95–96	Unk
		Custer	95	Unk
		Elmore	94	Unk
		Idaho	94–96	Unk

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Lemhi	95	Unk
		Shoshone	94	Unk
	L. minutus	Boise	96	E
		Custer	95	Unk
		Idaho	95	Unk
		Lemhi	95	Unk
	L. obtusus	Custer	95	Unk
	M. paucipunctella	Blaine	89	Unk
		Boise	89	Unk
		Bonner	87	Unk
		Idaho	87	Unk
		Latah	87–88	Unk
		Lemhi	87–89	Unk
		Nez Perce	87	Unk
	S. jugoslavica	Blaine	87	Unk
		Camas	90	E
		Gooding	90	E
		Jerome	87–89	E
		Lincoln	87–89	E
	T. virens	Butte	95	Unk
		Lemhi	95	Unk
INDIANA	*A. zoegana*	Elkhart	96	N
	C. achates	Elkhart	96	N
	L. minutus	Elkhart	96	N
MINNESOTA	*A. zoegana*	Becker	92–96	R
		Clearwater	92–93, 96	E
		Otter Tail	91	E
		Polk	92–96	E
		Washington	91, 93–94	E
	B. fausti	Becker	92	N
	C. acrolophi	Beltrami	96	R
		Washington	96	R

Table 1. Continued.

State	Agent	County	Year/years released	Status
	C. achates	Becker	94, 96	N
		Washington	95–96	R
	L. minutus	Becker	94, 95	E
		Otter Tail	95	E
	L. obtusus	Washington	95	N
	M. paucipunctella	Becker	92	R
		Otter Tail	92	R
		Washington	91–92	R
	T. virens	Clearwater	94, 96	R
MONTANA	*A. zoegana*	Broadwater	94–95	E
		Carbon	88–93	E
		Fergus	95	Unk
		Flathead	88–92	E
		Gallatin	88–96	E
		Jefferson	93	E
		Lewis & Clark	89	N
		Madison	97	N/A
		Mineral	93	Unk
		Park	93, 95–96	E
		Powell	89, 92	E
		Richland	93	Unk
		Sweet Grass	94	E
		Wheatland	92	E
	B. fausti	Broadwater	93–94	N
		Gallatin	92–93	N
		Lewis & Clark	92	R
		Park	94	N
		Sweet Grass	93	N
	C. acrolophi	Gallatin	93, 96–97	R
		Jefferson	93	N
	C. achates	Brodwater	94–95	E
		Gallatin	89–96	E

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Jefferson	94	E
		Madison	4	R
		Missoula	91	E
		Park	94–96	E
		Powell	93	E
		Sweet Grass	94–95	E
	L. minutus	Broadwater	94–97	E
		Carbon	97	N/A
		Gallatin	91–97	E
		Jefferson	97	N/A
		Lewis & Clark	91–92, 95, 97	E
		Madison	95, 97	R
		Missoula	95, 97	Unk
		Park	92, 95–97	E
		Powell	91, 95, 97	E
		Stillwater	94, 97	Unk
		Sweet Grass	92–96	R
	L. obtusus	Broadwater	93–95	E
		Gallatin	93–94, 96–97	E
		Park	95–96	R
	M. paucipunctella	Carbon	89	N
		Flathead	88–89	E
		Gallatin	88–89, 92	N
		Lewis & Clark	89	N
		Mineral	92	Unk
		Missoula	87, 89	Unk
		Powell	89, 91	N
		Ravalli	87	E
		Silver Bow	92	N
	P. medullana	Gallatin	93	N
	P. inspersa	Gallatin	90–94	N
	S. jugoslavica	Broadwater	96	N

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Fergus	90	Unk
		Lewis & Clark	88–91, 95–96	E
		Mineral	88–89, 93	Unk
		Powell	89	N
		Sweet Grass	93–95, 97	R
	T. virens	Gallatin	93–96	R
		Park	93–94	N
NEVADA	*A. zoegana*	Holt	91–95	R
		Pierce	95	N
	X b. fausti	Holt	94	R
		Madison	93	N
	C. achates	Holt	96	N
		Pierce	95–96	N
	L. minutus	Holt	92	N
		Pierce	94–95	E
	L. obtusus	Holt	95	N
	X m. paucipunctella	Antelope	93	R
	S. jugoslavica	Pierce	93, 95–96	N
	T. virens	Holt	96	N
	A. zoegana	Washoe	93, 95	N
		White Pine	94	N
		Eureka	97	N/A
	C. achates	White Pine	95	N
	L. minutus	Washoe	96–97	R
		White Pine	96–97	R
	S. jugoslavica	Washoe	94, 97	N
NORTH DAKOTA	*A. zoegana*	Kidder	91	D
OREGON	*A. zoegana*	Baker	92	E
		Deschutes	93	E
		Jackson	93	E
		Wallowa	91	E
	C. acrolophi	Deschutes	94	R

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Hood River	94	N
	C. achates	Deschutes	94, 96	E
		Hood River	94	R
		Jefferson	95, 96	R
		Morrow	95	R
	L. minutus	Deschutes	92	E
		Hood River	92	E
		Union	94	E
		Wasco	94	E
	L. obtusus	Deschutes	94, 95	N
		Hood River	94, 95	N
		Jefferson	94, 95	N
		Wallowa	94	N
	M. paucipunctella	Deschutes	87, 89	E
		Hood River	87	E
		Jefferson	87, 89	E
	P. inspersa	Hood River	95	N
		Lane	94	N
		Wasco	94	E
	S. jugoslavica	Deschutes	89	E
		Jefferson	89	E
		Morrow	89	E
		Umatilla	89	E
		Union	89	E
		Wallowa	89	E
		Wasco	87, 88, 89	E
	T. virens	Deschutes	93	N
		Hood River	93	E
		Lane	93	E
SOUTH DAKOTA	*A. zoegana*	Pennington	91, 93, 94	E
		Todd	92	N
	B. fausti	Pennington	94	N

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Shannon	94	N
	C. achates	Pennington	95, 97	E
		Shannon	94, 95, 96	N
	L. minutus	Pennington	96	R
		Shannon	95	E
		Tripp	97	N/A
	T. virens	Pennington	96	R
	S. jugoslavica	Shannon	94, 95, 96	E
		Todd	90	R
		Tripp	92	N
UTAH	*A. zoegana*	Cache	94, 97	E
		Utah	96	N
		Wasatch	94, 97	N
		Weber	92	N
	C. achates	Box Elder	97	N/A
		Davis	95	E
		Grand	95	N
		Utah	95, 96	N
		Wasatch	94	E
	L. minutus	Box Elder	97	N/A
		Cache	97	N/A
		Davis	96	E
		Grand	96, 97	N
		Utah	96	N
		Wasatch	97	N/A
	S. jugoslavica	Box Elder	97	N/A
		Davis	96–97	E
		Grand	97	N/A
		Utah	96	N
		Weber	93–94	E
WASHINGTON	*A. zoegana*	Spokane	91, 96–97	E
	C. acrolophi	Spokane	96	N

Table 1. Continued.

State	Agent	County	Year/years released	Status
	C. achates	Chelan	95	N
		Kittitas	97	N/A
		Lincoln	97	N/A
		Spokane	94, 96	E
		Stevens	95	E
		Yakima	94	E
	L. minutus	Benton	96	N
		Chelan	96	E
		Franklin	96	E
		Kittitas	96	E
		Okanogan	96	E
		Spokane	92	E
		Stevens	96	E
	L. obtusus	Pend Orielle	94	E
		Spokane	94, 96–97	E
	S. jugoslavica	Adams	90	E
		Asotin	89	E
		Benton	88–90	E
		Chelan	95–96	N
		Columbia	95	N
		Douglas	96	N
		Ferry	97	N
		Franklin	95	N
		Kittitas	88–91, 93–94	E
		Lincoln	89	E
		Okanogan	87, 91, 93, 96	E
		Spokane	88–90, 93, 96	E
		Stevens	91, 94–96	E
		Yakima	89–90	E
	M. paucipunctella	Columbia	96	N
		Kittitas	96	N
		Spokane	87, 95–96	E

Table 1. Continued.

State	Agent	County	Year/years released	Status
		Stevens	87, 96	E
WISCONSIN	*A. zoegana*	Iowa	91	N
WYOMING	*A. zoegana*	Johnson	94	E
		Lincoln	93	E
		Sheridan	95	E
		Teton	92	E
	B. fausti	Sheridan	95	N
	C. acrolophi	Johnson	93	N
		Lincoln	97	N/A
		Sheridan	96	N
	C. achates	Johnson	94–95	E
		Lincoln	96	N
		Platte	95	E
	L. minutus	Johnson	95	E
		Lincoln	95	E
		Natrona	91, 94	E
		Teton	92	N
	L. obtusus	Sheridan	96	R
	S. jugoslavica	Johnson	95	E
		Lincoln	95	N
		Natrona	94	E
	T. virens	Johnson	93	R
		Lincoln	95	Unk

E = Established and most sites are collectable.
R = Recovered populations are small or have not been consistently recovered.
N = The agent failed to establish.
Unk = The status of the agent is uncertain.
D = The site has been destroyed.

Table 2. Release dates for biological control agents of diffuse and spotted knapweed.

Agent	Year first released in USA	Released by	Year released by APHIS
A. zoegana	1984	MSU*	1988
B. fausti	1992	ARS	1992
C. acrolophi	1992	MSU*	1993
C. achates	1988	MSU*	1990
L. minutus	1991	MSU* (joint)	1991 (joint)
L. obtusus	1992	MSU*	1993
M. paucipunctella	1980	MSU*	1991
P. medullana	1984	MSU*	1993
P. inspersa	1988	MSU*	1991
S. jugoslavica	1983	MSU*	1990
T. virens	1992	MSU*	1993
U. affinis	1973	ARS	1988
U. quadrifasciata	self introduced**		1989

* Montana State University, Bozeman, MT.
** (Gillespie 1983).

and Wyoming. *Larinus minutus* is in Phase II in Colorado, Minnesota, Nebraska, and in Phase III in Idaho, Montana, Oregon, South Dakota, Utah, Washington and Wyoming. *Larinus obtusus* is in Phase II in Montana. *Metzneria paucipunctella* is in Phase III in Oregon, Washington and collectable in Montana. *Sphenoptera jugoslavica* is in Phase II in Idaho, Montana, South Dakota, Utah, and in Phase III in Colorado, Oregon, Washington, and Wyoming. *Terellia virens* is in Phase II in Oregon. *Urophora affinis* is in Phase III in Colorado, Michigan, Minnesota, Utah, and Wyoming. Idaho, Montana, Oregon, and Washington have completed redistribution of *Urophora affinis*. *Urophora quadrifasciata* is in Phase III in Colorado, Michigan, Minnesota, Nebraska, South Dakota, Utah, and Wyoming. Montana, Oregon, and Washington have completed redistribution of *U. quadrifasciata* (Lang et al. 1998).

Diffuse and spotted knapweed agents multiply at different rates. The large numbers of agents that are being redistributed reflect those agents that have adapted well and have high reproductive rates. APHIS redistributed 35,000 adult *Larinus minutus* from one FIS in Montana in 1997 which originated from a release of 795 adult weevils in 1991. In 1990 APHIS shipped approximately 400,000 adult *U. affinis* and *U. quadrifasciata* to seven states. Colorado Department of Agriculture Insectary in Palisades, Colorado in cooperation with APHIS has produced approximately 5000 adult *C. achates* from their knapweed garden each year in 1995 and 1996 (Lang 1995, 1996). The initial population was eight pair in 1992 and an additional release of 41 adults in 1993. Establishment has been successful in eleven of the thirteen agents from APHIS.

Further releases of diffuse and spotted knapweed biocontrol agents are now the responsibility of the states and the insectaries are under their management. APHIS has completed its goal of importing and establishing insectaries for biocontrol of spotted and diffuse knapweed in the appropriate states. Continued monitoring of establishment and natural spread of these biocontrol agents for spotted and diffuse knapweed needs to be continued. Monitoring of efficacy of individual and combinations of knapweed biocontrol agents needs to be done.

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